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Separation and Purification of Degradation Products from Spent *Auricularia auricular* Substrate Oxidized via Hydrogen Peroxide/Acetic Anhydride Using Polyamide Column Chromatography

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A spent *Auricularia auricular* substrate was oxidatively degraded using aqueous hydrogen peroxide/acetic anhydride to obtain a soluble portion (SP). The SP was sequentially eluted by using a polyamide-packed column with petroleum ether (PE), chlorine dioxide solution (CDS), ethyl acetate (EA), and mixed solutions of PE with CDS, and CDS with EA at different volume ratios, yielding 11 eluate fractions (F_1–F_{11}). In gas chromatography/mass spectrometry (GC/MS), 19 elution fractions (EF_1–EF_{19}) with similar chemical compositions were obtained. 136 compounds were identified in SP and EF1–19. Among these, seven compounds were completely separated: (r-(R*, R*))-2-acetoxy-3-hydroxysuccinic acid (AHSA), ribitol, tartaric acid (TA), 2-methyl-2-propenate-1,2-ethanediylester (MPEE), succinic acid (SA), acetoxyacetic acid (AAA), and dihydro-2,5-furandione (DHFDO). The purities of AHSA, ribitol, TA, MPEE, and DHFDO were 100.0, 100.0, 100.0, 96.0, and 99.0%, respectively, whereas those of SA and AAA were 100% after recrystallization. A series of carboxylic acids was enriched from SP and identified using a GC/MS system.

1. Introduction

Separated fine chemicals (FCs) using polyamide column chromatography (PACC) are used to produce biochemical sensor materials. If the separated FCs include bioactive compounds such as phenols, flavonoids, or polysaccharides, FCs are used mainly as biosensor elements for environmental or food safety. FCs with optical, electrochemical, or fluorescent properties are candidates for chemical sensing elements, for instance, in detecting metal ions, pH changes, or

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volatile compounds. FCs are mainly derived from natural substrates that have antioxidants or antimicrobial properties, as they are used to coat sensor surfaces to enhance stability, selectivity, or biocompatibility. FCs can be extracted using environment-friendly chemical separation methods that allow the recovery and reuse of solvents during chemical separation processes, reducing environmental impact and improving sustainability. Such methods enable environment-friendly sensor development, as sensing materials can be manufactured by sustainable methods.[1–4]

To identify and separate biosensor materials, we developed an effective separation and nondestructive method to analyze the molecular composition of organic matter in coal or biomass in previous studies.[5,6] In this method, we integrated fractional extraction, degradation, and column chromatographic techniques using a gas chromatography/mass spectrometry (GC/MS) system to avoid thermal decomposition and identify the original components in coal or biomass.[7] Using the separation and nondestructive method, amides, alkanes, alkanones, and aromatics can be enriched as group components,[8–15] and multiple esters and condensed arenes are separated as nearly pure compounds from coal or coal tar.[16–19] However, no researchers have separated and identified the degraded products from biomass via aqueous hydrogen peroxide/acetic anhydride (AHPO/AAH) and polyamide column chromatography has rarely been used for their separation and identification.[20–22]

In this study, we separated the soluble portion (SP) obtained from the oxidative degradation of spent *Auricularia auricular* substrate (SAAS) using polyamide column chromatography, yielding FCs. As SAAS is rich in lignocellulosic content and can be converted into biochar and carbon nanomaterials at a low cost and using an environment-friendly method, it is regarded as a candidate for sustainable sensor development.[23] Lignocellulose is oxidatively degraded by AHPO/AAH, converting SP in a high ratio. SP consists of numerous compounds, most of which are present in low concentrations and have similar boiling points.[24–26] Although many of these compounds serve as raw materials or synthetic precursors for biosensing chemicals,[27,28] their separation and purification in SP remain challenging. Effective SP separation methods are crucial to meet diverse, high-quality, and large-scale demands.[29] In this study, the structures of the FCs were confirmed by using mass spectrometry, and plausible separation mechanisms were proposed by analyzing the composition of compounds in the eluates. The separation process is proven to be operable, environment-friendly, and solvent-recyclable.

2. Methodology

2.1 Oxidative depolymerization procedure

Nine grams of SAAS was oxidatively degraded by AHPO/AAH at 45 °C for 16 h, followed by filtration to obtain SP and residue. SP was concentrated by solvent evaporation and extracted with acetone to obtain an acetone-soluble portion (ASP) and an acetone-insoluble portion (AISP). The ASP was then concentrated using a rotary evaporator and esterified with a CH_2N_2/diethyl ether solution (MASP) [Fig. 1(a)].

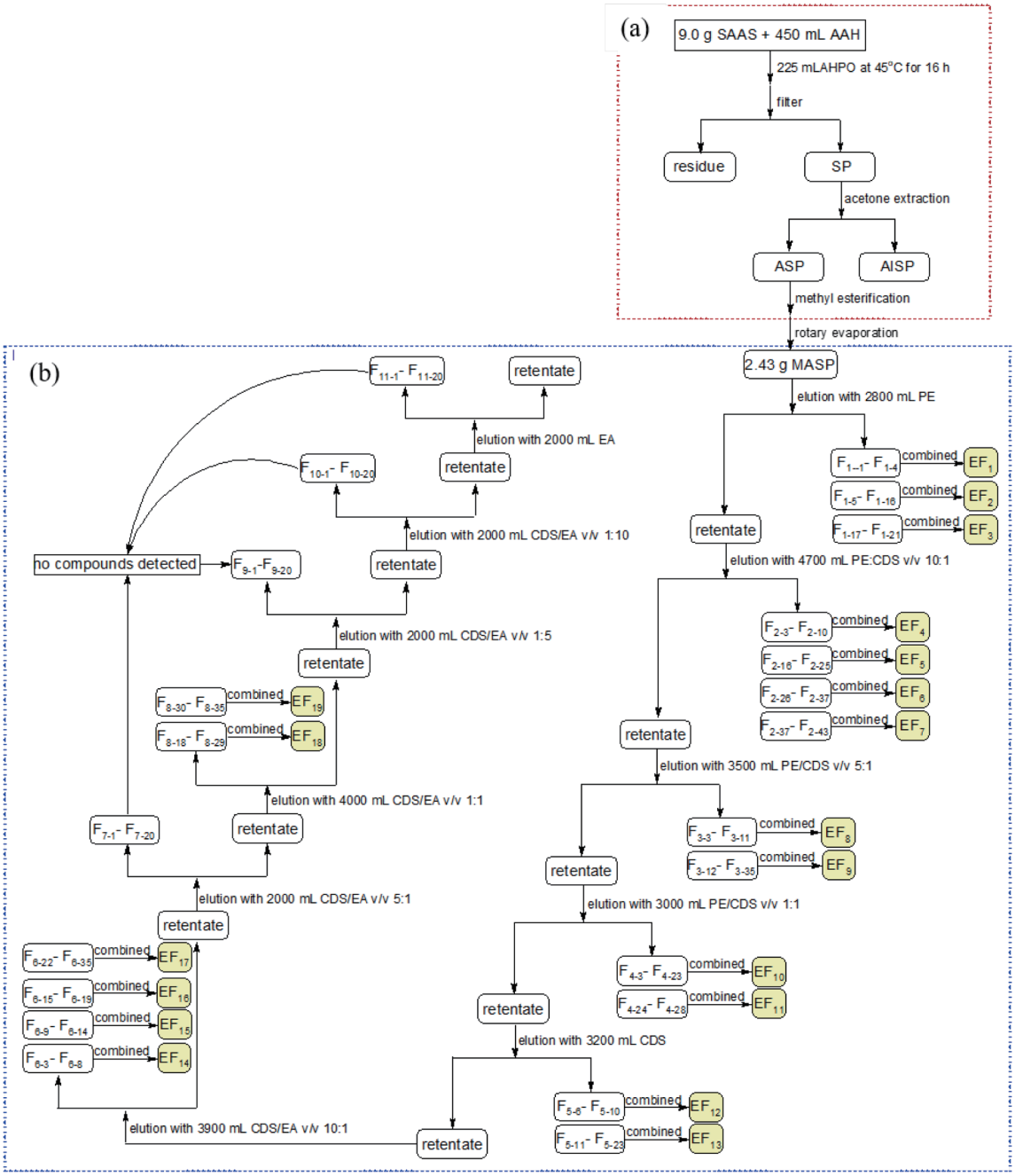

Fig. 1. (Color online) SAAS degradation and separation process of MASP.

2.2 Separation and purification procedure

MASP was separated by polyamide column chromatography, and a series of eluates were collected. In the specific operation, 2.43 g of MASP was loaded onto a column [1200 mm length and 25 mm inner diameter (ID)] with polyamide particles (<75 μm) [Fig. 1(b)]. The eluent flow rate was maintained at 1.8 mL/min, with each 100 mL eluent collected as a fraction (F_{n-i}). The eluates were rotary evaporated to near dryness and dried to a constant weight. The yield of each eluate (Y_E) was calculated as the mass ratio of the eluates (m_E) to MASP (m_{MASP}) using Eq. (1).

$$Y_E = \frac{\sum m_{Ei}}{m_{MASP}} \tag{1}$$

The eluents were reconstituted and analyzed using a Shimadzu QP 2010 Ultra GC/MS system equipped with a Rxi-5 column (30 m length × 0.25 mm ID, 0.25 μm film thickness) and a quadrupole analyzer with a m/z range from 35 to 500 operated in the electron impact of 70 eV. Data was processed with ChemStation software. The organic compounds were identified by comparing mass spectrometry results with the data of the National Institute of Standards and Technology (NIST) Mass Spectral Library. F_{n-i} containing the same compounds were combined into one elution fraction (EF_1–EF_{19}). A total of 19 EFs were obtained and recrystallized to improve the purity. Chlorine dioxide solution (CDS) was used as a solvent to obtain EFs.

2.3 External standard curve of compounds

To quantify (r-(R*,R*))-2-acetoxy-3-hydroxysuccinic acid (AHSA), ribitol, tartaric acid (TA), succinic acid (SA), acetoxyacetic acid (AAA), and dihydro-2,5-furandione (DHFDO) in SAAS, the external standard curves of concentration and peak area were drawn using the Origin plotting software. 2-Methyl-2-propenate-1,2-ethanediylester (MPEE) was not quantitatively analyzed because the standard is not commercially available. The content of the six compounds in SAAS was calculated using Eq. (2).

$$\omega = \frac{\sum c_i \times V_i \times f}{m \times 1000} \tag{2}$$

Here, c_i represents the concentration of the compound in each fraction (mg/mL), V_i represents the volume of each elution fraction (3 mL), f represents the dilution factor, m represents the quality of SAAS [g, dry and ash-free basis (daf)], and i represents the level of eluent.

2.4 Materials and reagents

The materials and nomenclature used in this study are detailed in Table 1. Table 2 presents the proximate, ultimate, and group composition analyses of SAAS. Proximate analysis was conducted to determine the amounts of four primary components of a material, typically measured by mass (weight percent, wt%). These components were determined by heating the sample under specific and standardized conditions. Final analysis was carried out to determine the elemental composition of the material. In group composition analysis, the materials were broken down into its major polymeric components.[30]

Table 1
Materials used in this study and their abbreviations.

Abbreviation	Material
AAA	acetoxyacetic acid
AAs	alkanoic acid
AAs'	alkenoic acid
AAH	acetic anhydride
ADAs	alkanedioic acids
ADAs'	alkenedioic acids
AHSA	(r-(R*,R*))-2-acetyloxy-3-hydroxysuccinic acid
AISP	acetone insoluble portion
ATAs	alkanetricarboxylic acids
ATAs'	alkenetricarboxylic acids
ATAs''	alkanetetracarboxylic acids
AHPO	aqueous hydrogen peroxide
ASP	acetone extraction of soluble portions
BAs	benzoic acid
BTAs	benzenetricarboxylic acids
BTAs'	benzenetetracarboxylic acids
CDS	carbon disulfide
DEDMBD	diethyl 2,3-dimethoxybutanedioate
DHFDO	dihydro-2,5-furandione
DHMFDO	dihydro-3-methyl-2,5-furandione
EA	ethyl alkanoates
EAs	ethyl acetate
EF	elution fractions
FCs	fine chemicals
FS	fraction section
GC/MS	gas chromatography/ mass spectrometry
HC	hemicellulose
MAs	monosaccharide alcohols
MADs	malonic acid derivatives
MADAs	methyl alkanedioic acids
MASP	ASP esterified by CH_2N_2/diethyl ether solution
MPEE	2-methyl-2-propenate-1,2-ethanediylester
NADAs	normal alkanedioic acids
NCCs	nitrogen-containing compounds
OAAs	oxyalkanoic acids
OADAs	oxyalkanedioic acids
PADs	other compounds
PACC	polyamides column chromatography.
PAs	phenylacetic acids
PE	petroleum ether
RC	relative content
SA	succinic acid
SADs	succinic acid derivatives
SAAS	spent auricularia auricular substrate
TA	tartaric acid
SCCs	sulfur-containing compounds
SP	soluble portion

Table 2
Results of proximate, ultimate, and group composition analyses (wt%).

Proximate analysis			Final analysis					Group composition analysis		
M_{ad}	A_d	VM_{daf}	C_{daf}	H_{daf}	N_{daf}	$S_{t,d}$	O_{diff}	Cellulose	HC	Lignin
9.84	7.34	91.63	43.00	8.40	0.75	0.11	47.74	34.81	24.27	30.64

daf: dry and ash-free base; M_{ad}: moisture (air-dried base); A_d: ash (dry base, i.e., moisture-free base); VM_{daf}: volatile matter (dry and ash-free base); $S_{t,d}$: total sulfur (dry base), and diff: by difference; HC: hemicelluloses.

3. Results and Discussion

3.1 Y_E of eluates

The Y_E of different eluates is shown in Fig. 2. The total Y_E was 89.5%, and 10.5% was adsorbed on the packing as the unadsorbed fraction (spent adsorber) of a compound that becomes irreversibly adsorbed to the stationary phase (packing material) and cannot be eluted or recovered during the separation process (dead adsorption). Among them, Y_E in the solution of 5:1, 1:5, and 1:10 (CDS:EA in volume) and EA was less than 2%, and no compounds were detected or identified in the eluates. Y_E of 1:1 in the volume of the CDS and EA mixture was the highest in the 11 eluates.

3.2 Compositions of MASP and eluates

136 compounds from MASP and eluates of solutions of PE, PE:CDS (10:1 in volume), PE:CDS (5:1), PE:CDS (1:1), CDS, CDS:EA (10:1), and CDS:EA (1:1) were identified with GC/MS and classified into 40 group components. 65 and 98 compounds were detected in MASP and the eluates, respectively. 38 compounds in MASP were not detected in any of the eluates. There were two main reasons, one was that the RC of several compounds such as octadecanoic acid, 3-hydroxy-3-methylbutanoic acid, methylmalonic acid, and 2-methylpentanedioic acid showed low content, and the 2-nitro-propanoic acid content was less than 0.1%. The second reason was that the rest of the 38 compounds, including oxalic acid, malonic acid, 5-oxohexanoic acid, and 3-acetoxy-3-hydroxypropionic acid, had higher polarity and were not easily eluted as spent adsorbers.

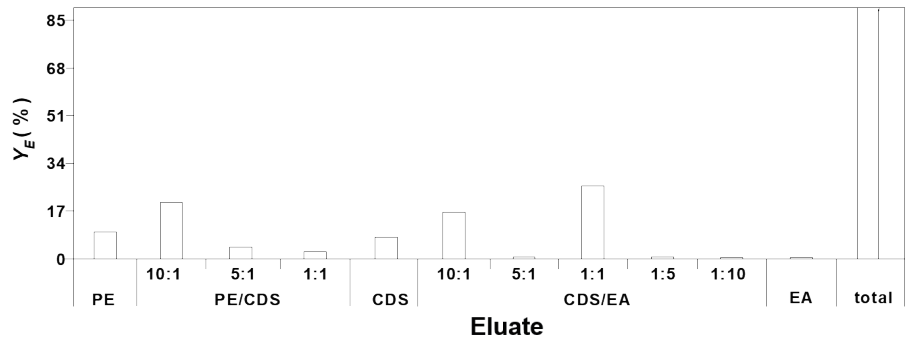

Fig. 2. Y_E in different eluates.

The RC of the compounds detected in EF_6, EF_9, EF_{11}, and EF_{19} was higher than 99.0%, whereas the RC of compounds in EF_{13}, EF_{15}, and EF_{17} was higher than 92%. In CDS, it reached 100% after recrystallization. AHSA, ribitol, TA, MPEE, SA, AAA, and DHFDO in MASP were successfully separated using the mixtures of PE:CDS (10:1), PE:CDS (5:1), PE:CDS (1:1), CDS, CDS:EA (10:1), and CDS:EA (1:1) as the mobile phase and polyamide as the stationary phase, and their structures were preliminarily identified using the GC/MS system (Fig. 3).

The contents of the six pure compounds in SAAS, except MPEE, are shown in Fig. 4.

Several compounds in MASP were not successfully separated and purified, but they were enriched. The RC of 2,3-dihydroxypropyl acetate, 2-hydroxy-2-methylbutanedioic acid, acetoxymalonic acid, and 2-oxo-pentanedioic acid was increased by 10 times. The RC of the other compounds also increased significantly (Fig. 5). The results show that polyamide column chromatography effectively separated and purified compounds in MASP, but the compounds enriched needed to be separated to obtain pure products.

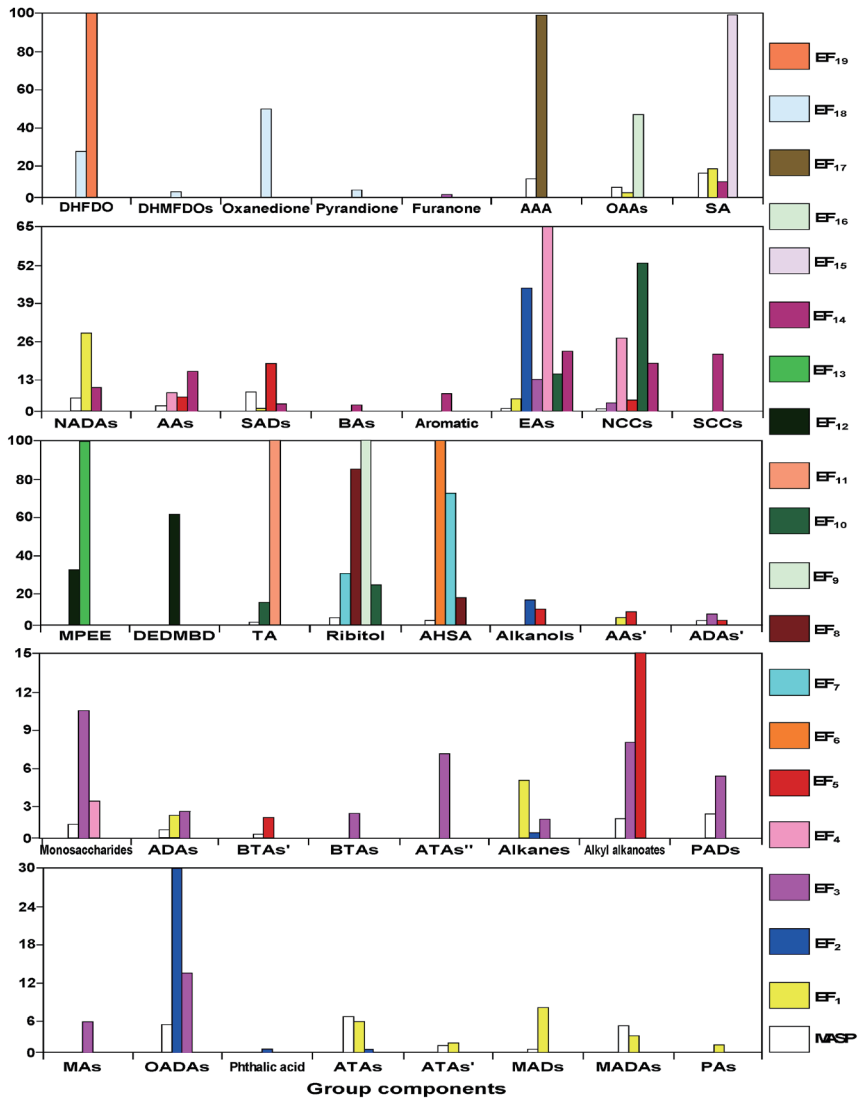

Fig. 3. (Color online) Distribution of group components in MASP and EF_1–EF19.

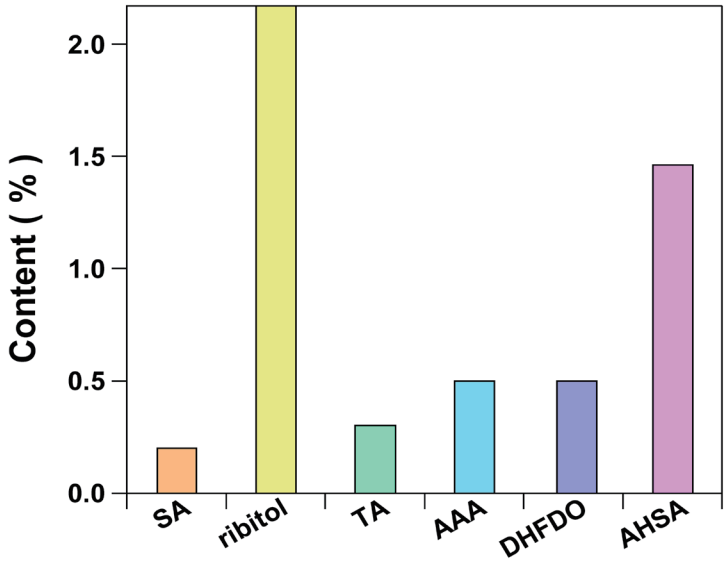

Fig. 4. (Color online) Content of six pure compounds in SAAS.

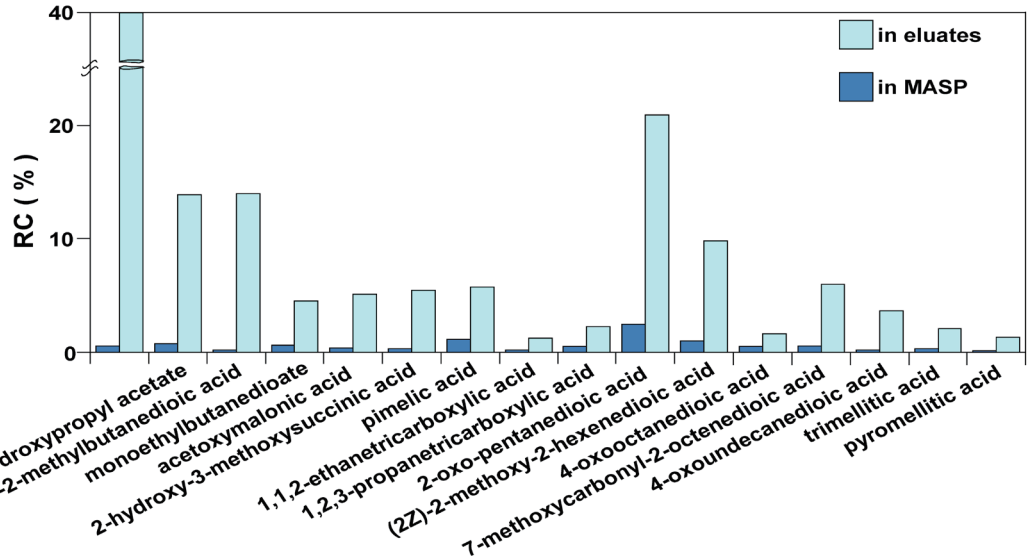

Fig. 5. (Color online) RC of compounds in MASP and eluates.

3.3 Separation mechanisms

Polyamide is a macromolecular compound with a repeated amide bond (–CO–NH–) in its structure. On the amide group, O and N bind to protons in the acidic medium and carry a positive charge, forming an electrostatic attraction and adsorbing anions in the solution. In column chromatography, the general principle of elution separation is “like dissolves like.” The elution principle of PE is to form a dispersion force with the nonpolar or weakly polar compounds

in MASP, which are dissolved and eluted.[31] When CDS is used as the eluent, CDS forms a π–π interaction with the double-bond-containing compounds adsorbed on the polyamide, so that the compounds are dissolved and eluted.[32] When EA is an eluent, it forms an orientation force with more polar compounds in MASP, which are solubilized and eluted more easily.[33]

Polyamide forms hydrogen bonds with phenols, carboxylic acids, quinones, flavonoids, esters, and other hydroxyl-rich compounds that are adsorbed to substrates. The more hydrogen bonding groups, the stronger the adsorption force.[34] In general, polyamide column chromatography is a double-phase chromatography because its molecules contain both hydrophilic groups (–CO–NH–) and lipophilic groups (>CH_2). When polar solvents are used as mobile phase, the alkyl group in the polyamide acts as a non-/weakly polar stationary phase, and the chromatographic behavior is similar to reverse phase chromatography. When using non-/weakly polar solvents in the mobile phase, the –CO–NH– group in the polyamide acts as a polar stationary phase, and the chromatographic behavior is similar to that of normal phase chromatography (Fig. 6).

In this study, we used polyamide column chromatography as normal phase chromatography to separate MASP. Weakly polar compounds in MASP were easily eluted, although polar

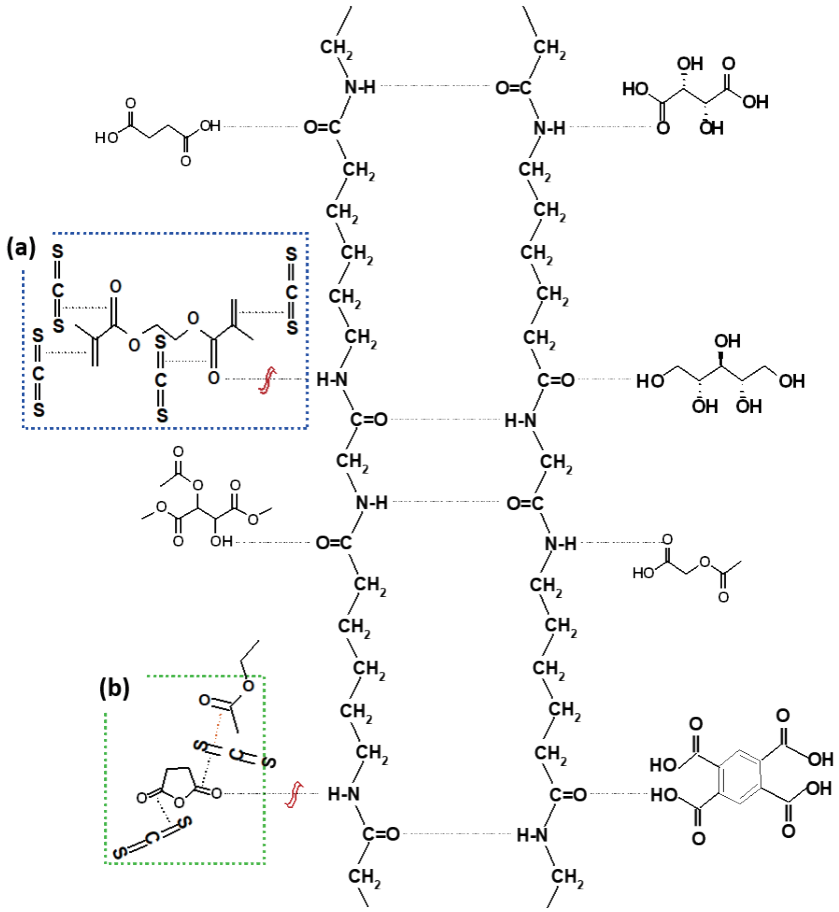

Fig. 6. (Color online) Mechanism of separation of compounds in MASP in polyamide column chromatography.

compounds are not easily eluted in general and are adsorbed to substrates. Oxalic acid and malonic acid in MASP were not detected in the eluates, but they became dead adsorbents. AHSA, ribitol, TA, MPEE, SA, AAA, and DHFDO formed new hydrogen bonds with –CO– and/or –NH– of polyamide molecules and were adsorbed. When elution was performed using weakly polar organic solvents, the compounds adsorbed on the polyamide were dissolved in different eluents in accordance with the different intermolecular forces formed and thus were eluted step by step. As shown in Figs. 6(a) and 6(b), when CDS was an eluent, the double bonds in its structure underwent π–π interaction with the double bonds in the MPEE structure, thereby breaking the hydrogen bonds formed by MPEE and –NH– and being eluted. When the mixture of CDS:EA (1:1) containing double bonds in the structure was an eluent, it formed a strong π–π interaction with the double bonds in the DHFDO structure, thereby breaking the hydrogen bond formed by DHFDO and –NH– and being washed away.

3.4 Applicability of method

The method developed employs PACC to separate SP obtained from oxidative degradation. The method can be applied to other natural substrates or biomass that contains lignocellulose-containing biomass. In the degradation step, an established method (AHPO/AAH) was employed for the oxidative degradation of lignocellulose to obtain SP. The degradation and subsequent separation method based on PACC is effective for separating and purifying compounds, including carboxylic acids, phenols, flavonoids, quinones, and esters, owing to the strong hydrogen bond interaction between the polyamide's –CO–NH– groups and the hydroxyl-rich compounds. Since many natural substrates and degraded biomass products contain these compounds, the method is applicable for their isolation.

The separation process in the method used in this study is easily operable, environment-friendly, and solvent-recyclable. Furthermore, the method allows for the enrichment of compounds that can serve as raw materials or intermediates for biosensors, such as succinic acid and ribitol. However, the process of the method is complex and might yield a low single-component content for other compounds. To obtain the maximum efficiency and pure products, further development of separation and purification methods with higher selectivity is required.

4. Conclusions

A series of organic compounds obtained by the oxidative degradation of SAAS using AHPO/AAH were separated and identified by polyamide column chromatography and GC/MS. The strong hydrogen bond interaction between –OH in organic compounds and –CO–NH– in polyamide separates organic compounds in biomass degradation. Seven compounds were successfully separated and purified. The polyamide used was recycled chemically,[35] and the organic solvents were recovered, which verified the developed method to be an effective, environment-friendly, and pollution-free process. Many compounds obtained by separation are important organic chemicals used as raw materials or intermediates for biosensors. For example, succinic acid as a C_4 platform compound can be used to synthesize 1,4-butanediol,

tetrahydrofuran, γ-butyrolactone, and other four-carbon chemicals, which are widely used in medicine, food, and the chemical industry. In addition, more than 200 chemical products produced using benzene can be replaced with succinic acid as a raw material and a substitute for petrochemical products. Ribitol and 3-hydroxypropionic acid are also potential compounds produced by biomass degradation. However, the process is complex, producing a low single-component content. Separation and purification methods with higher selectivity needs to be developed, requiring the design and development of supporting equipment and devices for efficient resource utilization.

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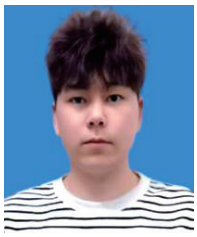

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